Salinity tolerance and osmoregulatory function of mannitol in Danish ecotype of *Saccharina latissima*

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Abstract

The use of brown algae in numerous commercial and industrial applications has resulted in extensive research into the ecology and ecophysiology of these macrophytes. Brown algae are cultivated for human consumption, feed supplement in aquaculture, and extraction of commercially-relevant chemical compounds, and have been identified as a potentially effective means of bioremediation of nutrient-polluted waters. *Saccharina latissima* (formerly *Laminaria saccharina*), a common coastal kelp in polar and temperate waters, is of particular interest to researchers due to its wide distribution along arctic and temperate coastlines, suggestive of robust traits. Ecotypic diversity in *S. latissima* is associated with a broad tolerance to various abiotic stressors, including salinity, the fluctuation of which has the effect of imposing osmotic stress on aquatic organisms. Besides the osmoregulatory function of inorganic ions, it has been shown that mannitol, a simple monosaccharide polyol, may fulfill a similar role in osmotic adjustment, albeit more effectively as a compatible solute. In this study, juvenile sporophytes of *S. latissima* were removed from a line cultivation site in the Danish Limfjord and treated at variable levels of steady-state salinity (5-30 ppt) over a month-long period in order to determine optimal salinity for growth as well as to investigate the role of mannitol as a possible osmoprotectant. In addition to growth and survivorship, various parameters related to photosynthetic efficiency were measured using pulse-amplitude modulated (PAM) fluorometry. Exposure to low-salinity treatment resulted in total mortality within a week at the 5 ppt level; survivorship at the 10 ppt level was 20% by the end of the study. Elongation and relative growth rates were significantly impacted at lower salinities compared with the optimal range (25-30 ppt). Mannitol concentrations showed little variation across lower salinity levels, with the highest concentrations found at 25 and 30 ppt, which also differed significantly from the baseline (pre-treatment) concentration. However, all levels tended to show non-significantly higher concentrations of mannitol compared with that found in baseline measurements for the sampled population. PAM fluorometry revealed a drop in \( F_v/F_m \) across all levels compared with the baseline; overall, \( F_v/F_m \) values were found to be much lower than expected, both prior to the experimental phase and at its conclusion, suggesting a stressed starting condition. Pigment content also showed a significant drop at lower salinities when compared with baseline levels.

*Keywords:* brown algae, saccharina, latissima, laminaria, salinity tolerance, mannitol, osmoregulation, compatible solute, osmoprotector, physiology, growth, survivorship, photosynthesis

1. Introduction

The important ecological role of brown algae (Phaeophyceae) in the cold-water, coastal habitats where they form communities is well-documented\[1\] \[2\] \[3\]. Mariculture of these macroalgae for human consumption has long been established as an industry in several Asian countries (namely China and Japan\[4\]) and the potential for developing similar enterprises elsewhere, such as the United States\[5\] and Europe\[6\] \[7\] \[8\], represents a growing field of interest.
Besides their value as a food product in themselves, brown algae are also a high-yield source of alginates, which have wide applications in the food and pharmaceutical industries [9, 10, 11, 12], although the species from which these biopolymers are extracted are not currently cultivated for such purposes. Beyond this, many new uses for kelp are being explored by researchers and industry alike, including their potential as feed in aqua- and mariculture [13, 14], as sources for biofuel production [15, 16, 17], and as effective bioremediators in nutrient-polluted coastal waters [18, 19].

Saccharina latissima (Linnaeus) C.E. Lane, C. Mayes, Druehl & G.W. Saunders, a Laminarian formerly classified within the genus Laminaria [20], is a common, perennial species that features a wide distribution along coastlines of circumpolar regions. This kelp, as members of order Laminariales are commonly referred to, is currently being investigated as a candidate for large-scale cultivation in Danish waters [21]; besides being indigenous to Danish waters (which is an important consideration in such undertakings), S. latissima demonstrates a relatively high tolerance to several abiotic stressors, including salinity, insolation [22] and, to a lesser degree, temperature [23].

Salinity tolerance is a critical component of the physiological performance of any estuarine organism. Whereas salinity in the open ocean remains relatively stable (∼35 ppt), along coastlines and in estuaries, it can fluctuate significantly throughout the year (and over shorter time-scales) due to the diluting effects of freshwater discharge, surface evaporation, wind, and precipitation [24]. In the Danish Limfjord, annual mean salinities typically occupy the 20-30 ppt range, but on the whole, Danish coastlines exhibit a steady distribution down to a mean value of ∼7 ppt [25]. Consequently, understanding how a given species responds to abiotic stressors such as salinity, is a vital first step in establishing a viable model for developing a nascent industry around the species in question.

Any consideration of the effects of salinity on an organism fundamentally deal with that organism’s ability to osmoregulate under hypo- and hypertonic conditions—a process whereby cell turgor is maintained via some mechanism capable of altering the intracellular concentration of solutes. In brown algae, it has been shown that the principal osmolytes are inorganic ions [26, 27, 28]; however, under relatively stressful conditions, the burden of osmotic regulation may be shifted to other osmolytes which, unlike ionic salts, do not interfere with regular cell metabolism when highly concentrated in the cytosol. It has been shown that mannitol, a simple sugar alcohol, may fulfil this role as a compatible solute [29, 28, 30].

The purpose of this study was to investigate the effects of different steady-state salinities on both the growth parameters (laminar length and wet weight) and survivorship of S. latissima sporo- phytes, as well as tissue composition in terms of relative concentrations of mannitol (which is expected to play a significant role in osmoregulation); content of chlorophyll a and several other accessory pigments were also determined, serving as indicators of plant stress. It was hypothesized that specimens of S. latissima would show fastest growth and greatest yield under optimal salinity conditions for this particular Danish ecotype (∼25 ppt). Furthermore, the role of mannitol as an effective osmoregulatory agent was expected to be significant (i.e. the relative concentration of mannitol was expected to rise as a function of increasingly hyposaline conditions).

1.1. Saccharina latissima

S. latissima, like other kelp, features a distinct, heteromorphic reproductive cycle in which a macroscopic, diploid (2N) sporophyte stage (Fig. 1) alternates with a microscopic, haploid (N) gametophyte. In the low tidal pools and sublittoral zones that the species typically inhabits [20], sporophytes form dense, sessile communities ("kelp forests") in which individuals are secured to the substrate via a holdfast, from which extend a stipe and blade.
Kelp, in general, possess another unique feature in that primary growth occurs at the transitional area from stipe to lamina. It is here that meristematic cell division occurs, such that the youngest tissue is found at the base of the lamina instead of the tip.

In general, optimal temperature for cultivation of kelp has been identified as falling within the 10-15°C range [31, 32], and further studies on *S. latissima*, specifically, match the results of these findings [23]. As for irradiance, *S. latissima* exhibits the expected decrease in photosynthetic efficiency at high levels of insolation (which is often the case for communities in intertidal zones during low tide); incurred photoinhibitory effects, however, are brief, due to effective heat dissipation via the xanthophyll cycle activity [33, 34]. Unlike temperature, it is somewhat more difficult to identify optimal levels of irradiance for *S. latissima*, as adaptations to variable insolation throughout the year result in different optima: in winter months, sporophytes compensate for low levels of irradiance and short days by investing more heavily in photosynthetic apparatus. On the other hand, during the summer months when sunlight is abundant, the concentration of xanthophyll pigments in the laminar tissues grows, to counter the effects of oxidative stress [35]. Overall, for a species like *S. latissima*, water temperature is the deciding factor in its distribution on a georaphical scale; locally, other abiotic factors, including insolation and salinity, figure more prominently [36].

1.2. Osmoregulation

There are several factors affected by salinity which play a significant role in biological processes: water density, concentration of ions, and, most importantly, osmotic pressure. As a direct function of salinity, osmotic pressure dictates the water potential/electrochemical gradients responsible for the flux of both water and ions across the cell membrane [37].

Reduction in turgor pressure is an immediate consequence of exposure to hypertonic conditions, as water escapes the cell across semipermeable membranes. The cell wall structure of brown algae is unique in that cellulose—which typically dominates the cell wall composition in higher plants—is
found in relatively low proportions (below 10%) [38]. Instead, matrix polysaccharides—including fucoidans and alginites—and phlorotannins feature prominently in the cell wall makeup. Despite the distinct structure of the cell wall, the cells of brown algae are still susceptible to plasmolysis under excessive water loss, a condition in which the cell membrane contracts to such a degree as to tear away from the cell wall causing irreparable damage. Less acutely, maintenance of cell turgor is important for continued cell growth [39].

Regular cell metabolism and function relies on the exchange and balance of several key inorganic ions, notably Ca$^{2+}$, Na$^+$, Mg$^{2+}$, K$^+$, and Cl$^-$ [26, 37, 40]. Active transport of these ionic osmolytes across the cell membrane represents the basic response of the cell to osmotic pressure in hyper- and hyposaline conditions. As such, for any organism that must contend on a regular basis with the osmotic stresses imposed by variable salinity, the ability to osmoregulate effectively and efficiently depends in large part on how intracellular ions are balanced.

In response to large fluctuations in salinity, the build-up of high concentrations of ions in the intracellular space may result in toxicity to regular enzymatic and ribosomal activities [41, 37] in addition to affecting membrane permeability (further exacerbating the initial condition) [42]. Prevention of the deleterious effects caused by excess ions relies, in part, on their storage in the vacuole, although this simply shifts the problem to within the cell by creating a new osmotic gradient between the vacuole and cytosol. This observation has led researchers to speculate on the potential osmoregulatory role of several compounds in counteracting the effects of excess inorganic ions in the cell and, in some cases, acting as primary osmolytes themselves.

1.3. Mannitol as a compatible solute

In the brown algae, mannitol (Fig. 2) and β-1,3-glucan laminarin are the main forms of carbon storage [43] and references therein], with mannitol making up 20-30% of the dry weight in some species [29]. Subsequent studies have been carried out to assess whether any relationship exists between mannitol levels and exposure to osmotic stress and, indeed, in S. latissima and several other species of brown algae, intracellular concentration of mannitol have been shown to vary as a direct function of salinity [44, 45, 29].

There are several good arguments in favor of an organism leveraging a compound such as mannitol for use in osmotic adjustment of cell turgor. In contrast to the aforementioned inorganic osmolytes, mannitol is a compatible solute (or osmoprotectant)—a compound which can be accumulated in the cytosol without inhibiting regular metabolic processes [46]. At least one study has shown that in S. latissima, inhibition of regular enzyme activity was strongly impacted by excess concentrations of K$^+$, Na$^+$, Cl$^-$, and NO$_3^-$, whereas mannitol had little to no effect whatsoever [28]. As mannitol already constitutes one of the major forms of carbon sequestration in kelp species, the necessary metabolic pathways for its biosynthesis and degradation are already in place [43], and thus a certain efficiency is achieved in terms of improved versatility of an already ubiquitous cellular component. This position is further supported by evidence in the literature for mannitol’s role in various processes other than osmoregulation, namely as an anti-oxidant and respiratory substrate [47, 30] and references therein].

![Figure 2: Structure of mannitol (C$_6$H$_8$(OH)$_6$).](image)
1.4. Chlorophyll fluorometry

The ability to uninvasively measure various parameters related to photosynthetic efficiency and, by extension, plant stress, is made possible via pulse-amplitude modulated (PAM) fluorometry. The technique is predicated on the assumption that incident light absorbed by a photosynthetic surface experiences one of three fates: (1) photons may be absorbed by the light-harvesting complex and used to drive photosynthesis; (2) excess photons may be quenched via non-photochemical processes and dissipated in the form of infra-red light (i.e. heat); or (3), photons may be re-emitted as light of a longer wavelength (i.e. fluorescence). PAM fluorometry is a relatively simple procedure that is carried out on living plants, in which exposure of a dark-adapted sample to a series of increasingly powerful pulses of photosynthetically-active radiation makes it possible to not only evaluate the photosynthetic capacity of a plant, but also to distinguish between the activity of photochemical and non-photochemical processes.

It is important to note that of the two distinct photosystems involved in photosynthesis, photosystem II (PSII) contributes overwhelmingly (>95%) to the fluorescence signals registered by fluorometric devices; consequently, as far as photochemical processes are concerned, fluorometry data provides insight into the efficiency of processes primarily associated with PSII.

Three commonly-used fluorometric parameters are made reference to in this paper: $F_v/F_m$ (maximum quantum yield of PSII), NPQ (non-photochemical quenching), and ETR$_{max}$ (maximum electron transport rate). What follows is a brief description of the significance and derivation of each parameter.

$F_v/F_m$ is a function of two basic measurements, $F_o$ and $F_m$. $F_o$, or minimal fluorescence, is an initial fluorescence measure of a dark-adapted sample under non-actinic light (i.e. light which is not capable of driving photosynthesis). $F_m$, or maximum fluorescence, is subsequently measured by exposing the dark-adapted sample to a saturating light pulse. This has the effect of reporting a value for fluorescence in the absence of competition from photochemical quenching; moreover, additional mechanisms associated with non-photochemical quenching, such as dissipation of heat via the xanthophyll cycle, are not yet mobilized at this stage. Together, $F_o$ and $F_m$ can be used to calculate $F_v/F_m$:

$$F_v/F_m = \frac{F_o - F_m}{F_m}$$

(1)

$F_v/F_m$ is particularly well-suited in gauging overall photosynthetic performance of analyzed plant tissue. The typical value of $F_v/F_m$ has been found to fall within a narrow range (0.83-0.85) for healthy specimens of many plant species[48, 49]; however, baseline measurements are nonetheless necessary, as the observed trend does not hold across all species. Any measure of quantum yield that falls significantly below a species’ baseline is typically an indicator of plant stress and photoinhibition[50].

NPQ is calculated in a similar manner to equation (1), making use of different measured coefficients to produce a value representing the capacity of the plant tissue to dissipate excess energy in the form of heat:

$$NPQ = \frac{F_m - F_m'}{F_m}$$

(2)

The coefficient $F_m'$ in (2) is the dark-adapted maximum fluorescence; in contrast, $F_m$ denotes the maximum fluorescence at some point after the initial dark-adapted measure has been made. In practice, this refers to the measurements taken at subsequent saturation pulses in fluorometric analysis. Since the value for NPQ is always calculated with respect to the dark-adapted point, it is possible to infer something about the efficiency of a plant’s heat-dissipating mechanisms by observing the change in their activity from a relaxed state under dark-adapted conditions[50].
ETR (from which \( \text{ETR}_{\text{max}} \) is later derived) is calculated in the following manner:

\[
\text{ETR} = \Phi_{\text{PSII}} \times \text{PAR} \times 0.5 \tag{3}
\]

The coefficient \( \Phi_{\text{PSII}} \) in equation (3) is known as the effective quantum yield of PSII and is calculated in the same way as \( \frac{F_v}{F_m} \) in equation (1); however, unlike \( \frac{F_v}{F_m} \), which must be determined from a dark-adapted sample, \( \Phi_{\text{PSII}} \) is derived from minimum and maximum fluorescence values on subsequent saturating pulses. Pulse irradiance is denoted in equation (3) by PAR (photosynthetically active radiation), expressed in units of \( \mu\text{mol photons m}^{-2}\text{s}^{-1} \); ETR, therefore, adopts these same units. Derivation of \( \text{ETR}_{\text{max}} \) is achieved by plotting a rapid light curve from ETR data in a fluorometric trace and determining the maximum of a fitted curve (Fig. 3). The coefficient 0.5 accounts for the fact that only \(~50\%\) of PAR is absorbed by PSII. Thus, the value of \( \text{ETR}_{\text{max}} \) produced by a rapid light curve is a simple and useful estimate of a plant’s optimal photosynthetic capacity at a given level of irradiance (i.e. non-photoinhibitory illumination).

2. Methodology

2.1. Specimen collection and experimental setup

Juvenile sporophytes (individual length ranging from 5-50 cm) of \( \text{S. latissima} \) were collected from an open-water, long-line cultivation site in the Danish Limfjord estuary (Fig. 4) on March 5, 2014. The shipped specimens arrived at Roskilde University on March 10, where they were transferred to a communal holding tank with water salinity at 25 ppt and a temperature of 13 °C. The 25 ppt salinity point was chosen to match the annual mean salinity of the southern portion of the Limfjord estuary in which the samples were cultivated[25].

Following a brief period of overnight acclimation, specimens were examined for physical damage to the stipe and meristematic tissue. From among the undamaged lot, 120 individuals were selected and randomly assigned to one of 7 treatment levels (5, 10, 15, 17.5, 20, 22.5, 25, 30 ppt). Initial measurements were taken for laminar length and blotted wet weight; additionally, holes were punched \(~1.5\) cm from the base of the lamina for each individual in order to track laminar growth. All specimens were cut down to a maximum length of 15 cm. At this stage, visible epiphytes were also removed. Specimens were subsequently placed into 20 L aquaria (water at 25 ppt salinity and 13 °C) according to their assigned levels, with 5 pseudoreplicates per aquarium and 3 aquaria per level (in total, 15 specimens per level). The aquaria were arranged on racks under halogen lights, aerated, and loosely covered with transparent plexiglass sheets to prevent excessive evaporation and water loss due to spray (Fig. 5). Cold room ambient temperature was set to 10 °C and an initial 12:12 light-dark (LD) regime was set. Over an 8-day acclimation period, treatment levels were gradually adjusted to their final points; during this same period, the LD cycle was also gradually raised to 16:8 to provide optimal growth levels. Waterproof electronic temperature/irradiation loggers were randomly distributed across the 6 shelving units to provide continuous tracking of condition throughout the experimental phase.
Following acclimation, the experimental phase extended over a period of 5 weeks. Growth measurements were taken at the start and end of this period. Baseline fluorometry readings were also taken at the beginning of the experimental phase, with final measurements across all treatment levels made at the end. Salinity levels for all tanks were measured and adjusted on a daily basis throughout the experimental phase; water in the tanks was completely replaced 4 times at regular intervals. Following the conclusion of the experiment, samples were removed from treatment and stored at −20°C. In preparation for the mannitol and pigment assays, samples were freeze-dried, powdered with mortar and pestle, and transferred to labelled vials.

2.2. Growth metrics

Relative growth rates (g g\(^{-1}\) day\(^{-1}\)) were calculated assuming exponential growth\(^{[51]}\) per the following equation:

\[
RGR = \frac{\ln(WW_2) - \ln(WW_1)}{\Delta t}
\]

In equation (4), \(WW_2\) and \(WW_1\) stand for the blotted wet weights of a sample at the end and beginning of the experimental phase, respectively; \(\Delta t\) represents the number of days over which the experimental phase extended.

2.3. PAM fluorometry

Serving as a baseline for comparison, fluorometry readings were taken for all 15 specimens at the 25

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**Figure 4:** Sampling location of *Saccharina latissima* specimens in Limfjord, Denmark. General area of line cultivation sites in inner estuary marked in purple. Red outline in inset map shows area of detail.
Figure 5: Experimental setup in cold room. Individual specimens shown randomly grouped into aerated tanks, serving as experimental units.

ppt level at the beginning of the experiment. Fluorometric parameters ($F_v/F_m$, NPQ, ETR$_{max}$) were measured using an Imaging-PAM M-series chlorophyll fluorometer connected to a PC running the proprietary ImagingWin (version 2.40b) application in a Windows 8 environment. The fluorometric apparatus comprised a control unit (IMAG-C, Walz) connected to a CCD camera (IMAG-K, Walz) and an LED ring-array (IMAG-L, Walz). Specimens were dark-adapted for 30 min prior to fluorometric analysis, whereupon they were individually removed from their respective aquaria and exposed to a series of saturating pulses under actinic light. For both initial and final measurements, 3 laterally-displaced sampling areas were chosen at the midpoint between hole punches (Fig. 6).

2.4. Mannitol assay

Quantification of mannitol in sample tissues was carried out using an adapted procedure, described here in detail. Stock solutions used in the procedure were prepared as follows:

- working solution: 6.666 mL 0.3 M periodic acid and 3.2 mL 1 M sodium bicarbonate added to 90.133 mL MilliQ (deionized water)
- Nash reagent: 15 g ammonium acetate, 0.3 mL pure acetic acid, 1 mL acetylacetone, topped up to 100 mL with MilliQ
- 0.1 M rhamnose solution
- 100 µg/mL mannitol solution

First, mannitol was extracted by adding pulverized sample to a labelled plastic vial with 10 mL 50% aqueous solution of isopropanol. After a 15 min extraction period, vials were centrifuged for 5 min at 9000 rpm. Supernatant was carefully removed and poured into a temporary vial and a second extraction was performed per the same method, resulting in a total of ~20 mL extracted solution.

For each sample, 4 vials were prepared (1 blank, 3 replicates). 0.5 mL of extracted sample solution was added to each of the 4 vials. 0.25 mL of working solution was then added and the sample was incubated at room temperature for precisely 10 s, at which point 0.25 mL of rhamnose solution was added and the contents of the vial were briefly agitated. Next, the sample was held once

Figure 6: Sampling scheme used in PAM fluorometry analysis. General sampling area was chosen at the midpoint between earlier holepunches (A) and older ones (B). Expanded area shows false color image (in this case, a representation of minimal fluorescence of viewed tissues) recorded by fluorometric apparatus. Selected sampling areas across which fluorescence data was measured and reported are depicted by the 3 laterally-arranged circles.
more at room temperature for a second incubation period of 5 min, at the end of which either 0.5 mL Nash reagent (for replicates) or 0.5 mL MilliQ (for blanks) was added to the sample. A final incubation of 15 min followed with the sample submerged in a 50°C water bath and subsequently allowed to cool back down to room temperature. Finally, 20 mL MilliQ was added to each vial and spectrophotometric absorbance was measured at 412 nm.

2.5. Pigment analysis

Determination of pigment concentrations was carried out via spectrophotometric (SP) and high-performance liquid chromatography (HPLC) analyses.

Measuring at 480, 510, 630, 664, and 750 nm, SP analysis was used to determine content of Chl \(a\), Chl \(c\), and total carotenoids. First, \(\sim\)5 mg of pulverized sample was weighed out, the exact mass recorded, and then added to 5 mL of 90% acetone. Extraction occurred over a diel period after which individual samples were agitated with a vortex mixer, passed through a 0.45 \(\mu\)m syringe filter, and separated into 2 replicate vials. Samples were finally passed through the spectrophotometer.

Spectrophotometric measurements of Chl \(a/c\) and total carotenoids were used to determine total concentrations according to the following equations, appearing in respective order:

\[
\text{Chl}_a = [11.47(A_{664}-A_{750}) - 0.40(A_{630}-A_{750})] \times \frac{\text{Vol}_{\text{ext}}}{\text{mass}}
\]

\[
\text{Chl}_c = [24.36(A_{630}-A_{750}) - 3.73(A_{664}-A_{750})] \times \frac{\text{Vol}_{\text{ext}}}{\text{mass}}
\]

\[
\text{Car}_{t} = [7.6(A_{480}-A_{750}) - 1.49(A_{510}-A_{750})] \times \frac{\text{Vol}_{\text{ext}}}{\text{mass}}
\]

where \(A_x\) stands for absorbancy at a given wavelength, \(\text{Vol}_{\text{ext}}\) represents extracted volume (mL), and mass refers to dry weight (mg) of sample used for extraction.

HPLC analysis was carried out by lab technicians on 2 mL ethanol extracts of samples in order to determine specific concentrations of fucoxanthin, violaxanthin, and zeaxanthin, in addition to Chl \(a\) and Chl \(c\).

3. Results

For the following set of results, it is important to comment on the manner in which the results at the 5 ppt level have been treated. Rapid degradation of the specimens was observed at the 5 ppt level, necessitating early harvest of tissues in order to carry out fluorometric and pigment analysis. Consequently, direct comparison of these metrics at the 5 ppt level against results at higher salinities is not entirely valid. Nonetheless, these values have been included in the result figures, where they are distinguished in some manner (typically in the form of an unfilled and unconnected data mark).

3.1. Statistical analysis

With the aid of SPSS predictive analytics software, one-way ANOVA was carried out on all data sets, with the exception of survivorship results. Homogeneity of variance was assessed by means of Levene’s test (\(P > 0.05\)) and results were processed with ANOVA (\(P > 0.05\)); where homogeneity was not determined, a Kruskal-Wallis test (\(P > 0.05\)) was used instead. In the case of detection of a significant difference among the tested means, a post hoc multiple-comparisons test was performed to identify significant differences between individual means at different treatment levels.

3.2. Survivorship

Survivorship was determined on the basis of visual assessment of tissue integrity: specimens for which it would not have been possible to carry out any further analysis due to complete or partial degradation of the thallus were deemed dead. Mortality was heavily expressed at low levels of salinity, in which dead specimens comprised little more than a stipe with necrotic tissues having been dislodged.
as a result of mechanical stress from handing and tank aeration. No survivors were observed at the 5 ppt level and only 3 individuals survived at 10 ppt (corresponding to 20% survivorship). Survivorship was 100% at all other levels (i.e. ≥15 ppt). It should be made clear that these results provide no insight into the specimens’ ability to recover from stress-associated damage and mid- to long-term recovery potential, as all tissue was harvested at the end of the experimental phase.

3.3. Growth rates

Results for RGR (Fig. 7) show a drop-off to either side of the 25 ppt level (considered to be the typical salinity for this ecotype). Following detection of a significant difference (one-way ANOVA, F=11.324, P<0.05) among the group means for RGR, a post hoc multiple comparison test was performed. The 15 (p=0.004), 17.5 (p=0.014) and 20 ppt (p=0.027) showed significantly lower RGR compared with the 25 ppt level; similarly, the 15 (p=0.047) level showed a significantly impaired RGR compared with the 30 ppt level. No significant difference was measured among the top 3 salinities (22.5, 25, 30 ppt).

Elongation rate (cm day⁻¹) of specimen blade lengths showed a similar and expected pattern (Fig. 8) as that seen in the RGR results, with the levels at the lower-end of the salinity treatment faring poorly in comparison with the optimal salinities.

Significant differences (Kruskal-Wallis, P<0.05) were found among the group means for elongation; a pair-wise comparison showed the 15 (p=0.003), 17.5 (p=0.011) and 20 ppt (p=0.040) to have significantly lower rates of elongation compared with the 25 ppt level; the 15 (p=0.012) and 17.5 ppt (p=0.046) levels also showed significantly lower elongation rates than the 30 ppt level. No significant difference was measured among the top 4 salinities (20, 22.5, 25, 30 ppt). A side-by-side comparison of the two metrics (Fig. 9) suggests a close relationship between laminar elongation and increase in wet weight, with a notable exception at the 30 ppt level.

3.4. PAM fluorometry

Fluorometric results for F₅/Fₐ show very little difference among the treatment levels, apart from specimens at 10 ppt (Fig. 10). A significant difference between the group means was detected (Kruskal-Wallis, P<0.05) and the 10 ppt level was the only value to fall significantly below that of the others. However, when comparing treatment levels to the baseline F₅/Fₐ (measured to be 0.52 at 25 ppt at the beginning of the experiment), both the 10 and, surprisingly, 25 ppt levels were found to have a significantly lower F₅/Fₐ.

Calculated values for ETRₘₐₓ (Fig. 11) show no discernible trend among the different salinity levels. Due to very poor performance, no reliable results could be obtained for the 10 ppt level.
Figure 8: Elongation of blade (cm day$^{-1}$) of *Saccharina latissima* over a 32-day period at variable steady-state salinities (10, 15, 17.5, 20, 22.5, 25, 30 ppt). The figure shows average values for each level ± SE (n=3) obtained by measuring distance between pairs of holes punched in blade at the start and end of the experimental phase. Too few specimens were available at the 10 ppt treatment level (unfilled circle) to yield results of statistical significance, but the average value of intact specimens from one experimental unit is displayed for purposes of discussion. No data could be obtained for the 5 ppt level due to early and rapid degradation of specimens.

Figure 9: Relative growth rate (g g$^{-1}$ day$^{-1}$) and blade elongation (cm day$^{-1}$) of *Saccharina latissima* shown side-by-side as a comparison of growth metrics.

Significance testing (Kruskal-Wallis, P<0.05) confirmed this observation, yielding no indication of a difference among group means. A comparison of ETR$_{max}$ in the treatments against that of the original baseline, however, shows a significant drop across all levels.

NPQ results, shown plotted against $\Phi_{PSII}$ (Fig. 12), show rapid onset of quenching dynamics across all levels barring 25 ppt, in which a smoother curve is observed. At the 25 and 30 ppt levels, a levelling-off of NPQ can be observed, in contrast to the other salinities, in which the upward trend prevents estimation of maximum quenching.

3.5. Mannitol

Mannitol concentration varied within a relatively narrow range, between 83.7 mg g$^{-1}$ DW (± SD
Figure 12: Nonphotochemical quenching (NPQ) of *Saccharina latissima* after 32-day treatment at variable stead-state salinities (5, 10, 15, 17.5, 20, 22.5, 25, 30 ppt). Changes in NPQ are shown alongside changes in the effective quantum yield of photosystem II (Y(II) on the graphs but also designated Φ$_{PSII}$ elsewhere in this paper) as photosynthetically active radiation (PAR) is increased. Error bars show SD (n=3). Scale ratios for NPQ and Y(II) have been preserved at 1:2, respectively, across all graphs to ensure consistent representation of dynamics.
Figure 11: Maximum electron transport rate of photosystem II (ETR\textsubscript{max}) for specimens of Saccharina latissima after 32-day treatment at variable steady-state salinities (± SE, n=3). Solid line shows ETR\textsubscript{max} for baseline (± SE, n=3). Reliable ETR values could not be determined at the 10 ppt level due to deteriorated condition of tissues. The 5 ppt level (unfilled circle) is set apart due to early harvesting of specimens (day 8), at which point ETR values could still be measured.

3.6. Pigments (Chl a, Chl c, carotenoids)

Pigment analysis revealed an expected reduction in overall concentrations of Chl a/c and fucoxanthin as a function of lower salinity treatment (Fig. 14). For Chl a, the concentration of which varied between 0.23 mg g\textsuperscript{-1} DW at 10 ppt and 0.93 mg g\textsuperscript{-1} DW at 30 ppt, significant differences (Kruskal-Wallis, P < 0.05) were found between the 10 ppt level and the 22.5, 25, and 30 ppt levels (p=0.045, p=0.016, p=0.004, respectively). Otherwise, all levels in the treatment except at the 22.5, 25, and 30 ppt showed a significant drop from baseline concentrations.

Chl c and fucoxanthin concentrations were also observed to have significant drop-offs at the 10 ppt level. Concentration of Chl c ranged from 0.013 mg g\textsuperscript{-1} DW at 10 ppt to 0.058 mg g\textsuperscript{-1} DW at 30 ppt. Significance in the difference of means (one-way ANOVA, F=8.331, P<0.05) was observed between 10 ppt and the 15, 22.5, 25, and 30 ppt levels (p=0.033, p=0.020, p=0.004, p=0.009, respec-
Figure 14: Concentration of Chl a/c and fucoxanthin (mg g\(^{-1}\) DW) in tissue samples of *Saccharina latissima* after 32-day treatment at variable steady-state salinities (± SE, n=3). Extraction was carried out with ethanol and sample contents were analyzed via HPLC. Pigment content at 5 ppt level are representative of concentrations on day 8, resulting from early harvesting of rapidly degraded tissues. Baseline measurements for concentrations of Chl a (square), Chl c (circle), and fucoxanthin (triangle) are shown apart on the left-most side of the graph. The right scale maps exclusively to the Chl c concentrations represented by a dotted line, in order to better illustrate the change in concentration relative to the other pigments.

Consolidating pigment content results from SP (not reported herein) and HPCL analysis, ratios of Chl c : Chl a and fucoxanthin : Chl a were produced (Fig. 15). Although differences in the sensitivity of both analyses is immediately apparent, the actual ratio in the case of the chlorophyll comparison only varies by a few percent points across all levels (scales have been adjusted to match scope of variation), with the highest proportion of Chl c found at the 15 ppt level (~9%) in the HPLC results and at the 22.5 ppt level (~23-24%) in the SP results. Interestingly, SP analysis reveals a general increase in the proportion of Chl c to Chl a compared with the baseline, in contrast to the HPLC results which show the opposite. Total carotenoid content to Chl a appears to rise as a function of lower salinities (up to ~113% at 10...
ppt) according to the SP results, with little fluctuation between 17.5 and 30 ppt (between ∼80-85%). In general, the ratio of total carotenoids to Chl a is higher when compared to the baseline ratio (∼60%). Looking specifically at the concentration of fucoxanthin to Chl a in the HPLC results, no obvious trend is exhibited, although the ratio appears to rise to either side of the 20 ppt mark, varying between ∼121% and ∼154%. The ratios here also run higher than the baseline measurement (∼109%) for all levels except the 5 ppt.

4. Discussion

Growth and survivorship of *S. latissima* specimens, as observed in this study, were significantly affected by treatment at relatively low salinity levels. Survivorship fell in line with observations from a previous study, in which young sporophytes of *S. latissima* cultured at 10 °C over a 4-day period showed 100% and 24% mortality at 6 and 11 ppt salinity, respectively [57]; the results reported in the present study, in which average water temperature in the experimental units was measured at ∼13 °C, showed a 100% (after 8 d) and 80% (after 32 d) mortality at comparable salinities of 5 and 10 ppt, respectively. The cited study appears to have used a similar criterion for assessing mortality (i.e. tissue fluorescence). Had specimens at the 5 ppt level in the present study been examined on day 4, a similar mortality rate would likely have been found.

Instead, plants were only removed from treatment at a point when tissue degradation had become severe. Additionally, the higher mortality observed at the 10 ppt level in the present study can simply be explained as a function of longer exposure time.

Growth metrics looking at blade elongation and relative growth also produced results correspondent with those found in the literature. In one study [58], which looked at the growth response of 2 distinct populations of *S. latissima* as a function of salinity (3 levels: 21, 27, and 23 ppt), measurements were taken after a 3-week acclimation period at 8 °C. Optimal growth was observed at the 27 ppt level, with comparable rates in one of the populations at 33 ppt and slower growth in the second population at the same level. In both populations, exposure to 21 ppt was found to significantly stymie growth. In the present study, optimal growth was also observed at the comparable salinity of 25 ppt and although no significant difference was found between the 3 highest salinities (22.5, 25, and 30 ppt), the observed trend implies that growth most likely does peak in the vicinity of 25 ppt for this particular geographic variant.

Comparing the two growth metrics side-by-side, there appears to be a strong correlation between blade length and total wet weight. One exception is found at the 30 ppt level (with no statistical measure of the significance of the effect), where blade elongation appears to cease alongside a reduction in overall wet weight. One possible interpretation of this result may be related to the methodological approach of measuring the blotted wet weight of samples, in which intracellular water content would have a predictably strong impact. As environmental hypertonicity increases, despite the work done by mannitol and other osmolytes in maintaining cell turgor, a net loss of water may occur due to movement along the water potential gradient (i.e. across the cell membrane to the extracellular environment) which is not completely counteracted by subsequent osmotic stabilization. If the specimen in question is showing halted or reduced growth (as is suggested by comparing blade elongation at the 25 and 30 ppt levels), then it may be reasonable to suggest that the cumulative loss of water across all exposed cells in the specimen—however small it may be for any given cell—would account for the observed decrease in wet weight. Furthermore, as mentioned earlier in this paper, cell turgor is closely related to growth; given this fact, the proposed reduction in cell turgor may contribute to some degree to the reduced growth seen at the 30 ppt level. Investigation into cell wall elasticity tolerances could be carried out with accurate measurements of the change in cell turgor (pressure probe technique [59]) as a function of salinity, in order to better understand to what degree changes in
the intracellular volume contribute to osmoregulatory function.

There is one further observation to be made in regards to the discrepancy between elongation rate and relative growth at 30 ppt which further supports the claim of mannitol’s role as an osmoregulatory agent. The two roles of mannitol as both an osmolyte and source of carbon sequestration (and, by extension, a substrate for growth) are competitive ones: if mannitol is being relied upon to maintain cell turgor then it cannot be used for growth, and vice versa. It therefore follows that, in a species such as *S. latissima*, during periods of exposure to high salinity, mannitol represents a trade-off between improved tolerance to hypertonic conditions and growth. An investigation into this dynamic could easily be adapted from the present study by extending the salinity scale past the 30 ppt point.

Temperature is a very relevant factor in all of these studies and, as such, a difference of a few degrees can have a strong effect on the comparability of outcomes. In the present study, some difficulties were encountered in the early phase of the acclimation period—prior to commencement of the experimental phase. Having identified an optimal range of 10-15°C for *S. latissima* from a number of sources in the literature, the cold room in which the experimental units and specimens were stored was set to an ambient temperature of 10°C; however, in the first 4-5 days of the acclimation, water temperature across all tanks was monitored and found to vary from 11-18°C, most likely due to poor air circulation. At least one study [60] has convincingly demonstrated the effect of ambient temperature on growth in *S. latissima*, with a 50-70% reduction in measured traits at 20°C under controlled conditions. Furthermore, total disintegration of specimens was observed after 7 days of exposure to 23°C, with similar results reported elsewhere [58] at 24°C. There is good potential for a robust species like *S. latissima* to exhibit strong recovery rates from transient exposure to abiotic stressors, but the life stage of the specimen, length of exposure, and degree of stressor are important in determining just how well the plant is able to recover. The physiological significance of this potential source of error will be further explored in the following section, in which fluorometry results are discussed.

Unlike growth, for which direct comparisons are difficult to evaluate, Chl fluorometry results suggest a very significant level of stress across all levels of the salinity treatment, both prior to and following the experimental phase. The well-established measured value of $F_v/F_m$ which, for healthy specimens of many plant species, falls within a narrow range of 0.83-0.85, is somewhat higher than the reported values for *S. latissima*, which range from 0.7-0.75 [34, 61, 62, 56]. In the present study, a baseline measure of 0.52 for $F_v/F_m$ is, consequently, a strong indicator of photo-inhibited specimens at the time of measurement.

Ideally, additional initial fluorometry measurements should have been taken shortly after specimens were sampled at the collection site; under such circumstances, it is very likely that measured $F_v/F_m$ would have reproduced the higher values reported in the literature. Instead, baseline fluorometry was determined at the beginning of the experimental phase, following an 8-day acclimation period. This is potentially problematic given the aforementioned difficulties in regulating ambient cold room temperature. During the same period, a problem with one of the analog time switches (used to automatically control light-dark cycles in the cold room) resulted in half of the randomly-distributed experimental units being exposed to the effects of continuous irradiance. An assumption was made about the ability of the plants to recover sufficiently by the end of acclimation period which, given further consideration, may not hold. As a measure of photosynthetic capacity, $F_v/F_m$ provides one method of assessing recovery from stress such as that just described.

Looking at the effects on photosynthetic performance as a function of exposure to UV-B, one
study on sporophytes of *S. latissima* from Kongsfjord (Spitsbergen, Norway) concluded that 2 factors played a significant role in determining the capacity of an individual to rebound from UV-induced photoinhibition. On the one hand, age was found to have a pronounced effect on recovery: following a 2-hour exposure to 30 \( \mu \text{mol m}^{-2}\text{s}^{-1} \) PAR and an 18-hour recovery period, \( F_v/F_m \) in 6-month-old winter sporophytes (collected in May) was measured at 28% of the initial value, compared with 63% in older specimens with thicker laminae (age unspecified). However, in addition to age, response varied depending also on seasonal adaptation: the same experiment carried out with June-July specimens (i.e. specimens which had become adapted to Arctic summer conditions) showed a complete recovery of \( F_v/F_m \) to initial levels after an 18-hour recovery period. (It is important to note at this point that Spitsbergen lies at 78° N, compared with the Danish Limfjord which lies at ~56° N; seasonal variation of environmental conditions is more severe in the circumpolar regions, but the principle under consideration still applies.) Another study looking specifically at the age-dependent response of *S. latissima* to UV irradiance found that young sporophytes (the other two categories being zoospores and gametophytes) showed the greatest sensitivity to treatment: following a 48 h exposure to 40 \( \mu \text{mol m}^{-2}\text{s}^{-1} \) PAR, \( F_v/F_m \) in the specimens showed little recovery as much as 7 days later, with values around the 0.2 mark.

The sporophytes under examination in this study were both young (<<6 months) and fully adapted to Danish winter conditions. Consequently, the conclusion that may be drawn from the results of the above studies is that, depending on how long the sporophytes in the present study were exposed to not only elevated temperatures, but prolonged irradiation as well, recovery to a stable steady-state by the end of the acclimation phase would represent an unlikely scenario.

\( ETR_{\text{max}} \) results were, unfortunately, not very enlightening. No clear trend can be extracted from the data, as there are no significant differences at any of the levels. Although \( ETR_{\text{max}} \) could not be calculated for the 10 ppt level, values can reasonably be assumed to have been (at some earlier point in the study) significantly lower than at the higher levels, given the drop in \( F_v/F_m \) at 10 ppt. The high mortality rate observed at this level is evidence of significant structural damage to the tissue, in which case reduced photosynthetic capacity is to be expected. What can be said is that the electron transport chain is dependent on the proper functioning of the entire subset of integral proteins, chlorophylls, and carotenoids associated with PSI and PSII; thus, any disproportionate change in the concentration or proper functioning of any one compound would likely translate into some effect detectable via \( ETR_{\text{max}} \).

As a measure of the efficiency with which heat is dissipated, an increase in NPQ describes one of two processes taking place (or a combination thereof). Firstly, it is representative of the mobilization of photoprotective mechanisms, such as the xanthophyll cycle, the role of which is to prevent photoinduced damage from occurring in the first place. Secondly, some part of the NPQ trace may represent actual damage taking place, in the sense that absorbed energy is contributing to some degree to the formation of harmful reactive oxygen species. In light of this, the steeper and prolonged escalation of NPQ and rapid drop-off in \( \Phi_{\text{PSII}} \) at salinity levels below 25 ppt can be interpreted as not only an increased need for NPQ, but is also suggestive of the occurrence of harmful quenching.

One peculiar trend in the NPQ results is that seen at the 30 ppt level: as the number of available ("open") reaction centres decreases (seen as a gradual decrease in \( \Phi_{\text{PSII}} \)), the expectation is for NPQ to rise proportionally, which is not the case; NPQ plateaus rapidly. As seen at the 10 ppt level, NPQ may increase past the point at which photochemical quenching has ceased playing an active role: all absorbed energy is simply converted via NPQ processes. However, it is more difficult to interpret a decrease in \( \Phi_{\text{PSII}} \) for which NPQ remains more or less stable, representative of an energy deficit of...
sorts. Overall, the 30 ppt treatment has yielded some interesting results, in which case the disproportion in the dynamics of $\Phi_{PSII}$ and NPQ likely points to some real effect, which certainly warrants further investigation.

As for the 25 ppt level, the NPQ and $\Phi_{PSII}$ curves provide further evidence of healthy specimens: NPQ activity kicks in more slowly than at other levels and appears to reach an equilibrium with $\Phi_{PSII}$ at 1000 PAR. As has already been shown, specimens at all levels were already stressed going into the experiment, in which case it is probable that with healthier samples, the same dynamic would have been observed but with a significantly higher values for $\Phi_{PSII}$.

Looking directly to the mannitol assay results, margins of error were unfortunately too large to detect any significant differences for the most part. However, careful consideration of the data reveals that the results do appear to validate the expectation of mannitol serving an osmoregulatory function, as will now be explained.

A recent study elucidating a number of details related to mannitol metabolism in the filamentous brown algae, *Ectocarpus siliculosis*, identified regulation of mannitol-1-phosphate dehydrogenase (a catalyst associated with the first step of mannitol synthesis) in response to NaCl concentration [30]. At $\sim$32 ppt, relative expression was observed to be 3 times higher compared with exposure to $\sim$19 ppt. The comparison across species is not ideal, but the range of salinities matches quite well with the observed results in the present study: at 30 ppt there is a significantly higher concentration of mannitol compared with the 20 ppt level (incidentally, this was the only significant difference detected between the means of the different treatment levels).

More difficult to explain is the significant increase at the 25 ppt level (which is the baseline salinity). Values for $F_v/F_m$ are indicative of a greater level of stress at 25 ppt measured at the end of the experimental phase, compared with the baseline, which might suggest that the increase in mannitol is at least partially a response to stress; the potential role of mannitol as an anti-oxidant is cited elsewhere in this paper, which would be the expected function in this case, as osmotic stress would not be a factor. Another possibility is that the elevated mannitol levels at 25 ppt may be related to experimental error. The results in this study have shown that a good deal of activity occurs to either side of the 25 ppt optimum, which further suggests that changes in the osmotic environment around this point lead to significant physiological responses. During the experimental phase, it was not uncommon for salinities at the various levels to rise by 2-3 points in between adjustments, owing to the difficulties in accurately gauging salinity with a refractometer and, simply, evaporation of water from the tanks. If the population of *S. latissima* in this study is, in fact, particularly sensitive to changes around the 25 ppt region, then it is entirely possible that the periodic fluctuation of salinity level in the treatment could induce similar changes seen at higher, stable salinities. These effects could easily be investigated in a subsequent study in which the resolution of the salinity spectrum is higher around the species optimum.

In *S. latissima*, besides the major photosynthetic molecule Chl $\alpha$, there are several accessory pigments that contribute significantly to the overall pigment profile: Chl $\epsilon$, fucoxanthin, violaxanthin, and zeaxanthin [64]. In this study, Chl $\alpha/c$ concentrations were successfully determined using both SP and HPCL; using the same analyses, an attempt was also made to identify not only total carotenoid content (which takes into account additional compounds heretofore not mentioned, such as $\beta$-carotene), but also individual concentrations of the previously listed accessory pigments. Unfortunately, no meaningful data could be obtained for concentrations of violaxanthin and zeaxanthin using HPLC., although SP produced a data set for
total carotenoid content.

Prior to discussing and providing some insight into the significance of the results of the pigment analysis, a brief description of the basic physiological role of each pigment is in order. Chl \( a \) plays a central role in the conversion of photo-electric to photochemical energy, as the primary electron donor in photosystem II (PSII). Ancillary to the efficient operation of Chl \( a \), is the accessory light-harvesting pigment, Chl \( c \), a chlorophyll molecule—unique to marine algae—that serves the critical function of absorbing light as part of the antenna complex\(^{[65]}\).

Fucoxanthin and violaxanthin are two xanthophylls that, among other functions, play a significant role in counteracting the harmful effects of oxidation caused by excess light at and around the photosynthetic reaction centres, a process referred to elsewhere in this paper as non-photochemical quenching (in order to distinguish it from the quenching of photons via photosynthesis) \(^{[66, 67]}\). In this study, zeaxanthin was also targeted for analysis, as both it and violaxanthin represent the two end products of the xanthophyll cycle, a mechanism that represents an effective solution to the problem of NPQ. In non-photoinhibited tissues, a higher proportion of violaxanthin to zeaxanthin is typically found; as oxidative stress increases in the thylakoid membrane, excess energy is quenched through the conversion of violaxanthin to zeaxanthin, resulting in a de-epoxidation ratio in favor a higher proportion of zeaxanthin \(^{[67]}\). As such, relative measures of each compound would have been a useful indicator of photoinhibition and overall plant health.

Together, Chl \( a/c \), fucoxanthin, and violaxanthin have been identified in \( S. \) \( latissima \) as forming a cohesive, fucoxanthin-containing light-harvesting complex (LHCF)\(^{[68]}\), distinct from the LHCs in other higher plants. Comparison of the concentrations of each pigment, whether temporally or relative to one another, thus, provides some insight into several factors including photosynthetic capacity and NPQ potential. Of interest, too, are the inferences that may be made about how a given species responds to abiotic stress by considering which mechanisms are prioritized over others and the manner in which resources are reallocated under such conditions.

Looking at the results from the pigment analysis, a very obvious discrepancy appears between the changing concentrations of chlorophylls and carotenoids and the corresponding fluorometric measurements. Given the data from the end of the experimental phase, one would expect that values for \( F_v/F_m \) would closely match the reduction at all levels of Chl \( a/c \), which is not the case: no significant or apparent change was measured in \( F_v/F_m \) between 15 and 30 ppt.

A possible cause for this unexpected result may stem from the manner in which data was obtained during Chl fluorometry analysis versus pigment extraction. Earlier in this paper, a description is provided of how fluorometry was performed: to briefly recapitulate, 3 sampling areas were chosen along a crosswise section of the blade. From these points, values for all fluorometric data was averaged. However, pigment analysis was carried out on the entirety of the harvested blade, the assumption being that the areas sampled during fluorometric analysis were representative of a homogenous tissue structure across the whole blade. The assumption is likely flawed, owing to the fact that primary growth is localized at the base of the blade; older parts of the plant might not, under fluorometric analysis, provide a representative state of the specimen as a whole. One solution would be to sample over a greater area (which produces more data but involves no extra work other than specifying the sampling spots in the software environment), and at several points along the length of the blade to produce a better fluorometric profile of the entire specimen. Tissues could then be harvested in parts and labelled according to which fluorometric measurements they correspond to.

Taking into consideration the pigment ratio re-
sults, the difference in sensitivity between SP and HPCL is apparent. A sharp increase in the proportion of Chl c to Chl a is observed at the 15 ppt level in the HPLC results, which is surprising given the otherwise very similar ratios at all other levels. If any change could be expected, it would more likely be a reduction in Chl c to Chl a at lower salinity levels, reflecting a reduced density of Chl c in the antenna complexes as a means of mitigating photoinhibition. An increase in the proportion of carotenoids at lower salinity levels (SP) is also suggested by the data, which would make sense given their important role in NPQ and scavenging of reactive oxygen species; of course, that is not to say that the role of Chl a is less important, only that the priority may shift away from photosynthesis and growth towards mechanisms associated with preventing the tissues from dying. Given that the proportion of fucoxanthin to Chl a does not appear to shift dramatically at lower salinities, the carotenoid ratio may in fact represent de novo synthesis of xanthophylls in response to increasingly stressful conditions.

In this study, the effects of salinity treatment on the growth, mannitol concentration, and pigment composition of *S. latissima* were determined with varying degrees of success. Although mannitol results were largely inconclusive, those results which were significant, along with supporting evidence inferred from growth rates, contribute to an overall case in support of its secondary function as an osmolyte. A number of suggestions related to methodological improvements have been outlined in the preceding discussion, and may prove useful for subsequent investigations along the same lines of inquiry.

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